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ABSTRACT

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The *Kappa* Opioid Agonist U-50,488H Antagonizes Respiratory Effects of *Mu* Opioid Receptor Agonists in Conscious Rats[1]

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ABSTRACT

The interactive effects of *mu* and *kappa* opioid receptor agonists on respiratory function were investigated following their i.c.v. injection into conscious rats. The highly selective *mu* receptor agonist [D-Ala^2,N-Methyl-Phe^4,Gly-ol] enkephalin (DAMGO; 1.2–10 nmol) and the relatively selective *mu* agonist morphine (20 and 30 nmol) significantly decreased arterial pH and PO_2, and increased arterial PCO_2 and blood pressure. Morphine and a low dose of DAMGO (1.2 nmol) also significantly elevated respiratory rate. Heart rate was decreased by DAMGO and, depending upon dose, was either decreased (20 nmol) or increased (30 nmol) by morphine. The selective *kappa* opioid agonist U-50,488H (200 nmol i.c.v.), which by itself had no significant effect on either respiration or cardiovascular function, dose-dependently antagonized the acidotic, hypoxemic and hypercapnic effects of both DAMGO (2.5 nmol) and morphine (30 nmol). Furthermore, these *mu* antagonistic properties of U-50,488H were blocked completely after pretreatment with 25 nmol of the highly selective *kappa* opioid antagonist nor-binaltorphimine. These results indicate that the antagonism of *mu* opioid respiratory depressant effects by U-50,488H is *kappa* opioid receptor mediated.

Narcotic opioid analgesics such as morphine are of tremendous therapeutic importance for the clinical management of pain. Unfortunately, these desired pharmacological effects are generally compromised by a number of associated liabilities, including potentially life-threatening cardiorespiratory depression (for review, see Yeadon and Kitchen, 1989; Shook *et al.*, 1990). Consequently, novel opioid or nonopioid analgesics free of these complicating side-effects have been continuously sought.

Following the recognition of multiple types of opioid receptors (*e.g.*, *mu*, *delta* and *kappa*) and the associated development of relatively selective opioid receptor ligands, a large number of investigations have been conducted to identify the relative involvement of each receptor class in the diverse pharmacology of opioids *in vivo*, and to potentially dissociate and/or correlate distinct opioid effects (such as analgesia and respiratory depression) through the use of ligands targeted at specific receptor types (for review, see Martin, 1983). Work with highly selective opioid ligands has also revealed distinct patterns of intriguing interactions among *mu*, *delta* and *kappa* opioid receptors, based upon the net *in vivo* responses recorded after administration of combinations of highly selective opioid agonists and antagonists. For example, *delta* opioid agonists appear to significantly potentiate or enhance antinociceptive (Vaught and Takemori, 1979; Porreca *et al.*, 1987; Heyman *et al.*, 1989; Jiang *et al.*, 1990) and autonomic (Sheldon *et al.*, 1989) effects of *mu* agonists. In addition, recent studies revealed that *kappa* receptor agonists can selectively antagonize the effects of *mu* agonists on bladder motility (Porreca and Tortella, 1987; Sheldon *et al.*, 1987, 1989), seizure threshold (Tortella and Holaday, 1986; Porreca and Tortella, 1987) and nociceptive responsiveness (Ramarao *et al.*, 1988) in rats. Moreover, characterizations of a variety of *mu* opioid ligands in several of these latter studies further revealed that *mu* opioid receptor agonists could be divided or grouped into two distinct subtypes based upon their manner of interactions with *kappa* receptor agonists.

Despite an intriguing preliminary report from Wood and coworkers (1982), interactive effects of selective *mu* and *kappa* opioid receptor agonists on central cardiorespiratory regulation have not yet been clearly and quantitatively established. Moreover, the impact of highly selective opioid ligands on respiratory status is somewhat ambiguous due to the limited selectivities of ligands used in earlier studies (Yeadon and Kitchen, 1989; Shook *et al.*, 1990). Therefore, the objective of the present study was to characterize cardiorespiratory responses to i.c.v. injec-

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[1] In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense (para 4-3, AR 360-5).

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ABBREVIATIONS: DAMGO, [D-Ala^2,N-Methyl^4,Gly-ol- enkephalin; nor-BNI, nor-binaltorphimine; EKC, ethylketocyclazocine.

tions of *mu* and *kappa* opioid ligands and to evaluate potential interactions between these two receptor systems on brain regulation of respiratory function in conscious unrestrained rats.

Methods

Chemicals. Morphine sulfate and U-50,488H were kindly donated by Dr. John D. Minna (National Cancer Institute, Bethesda, MD) and the Upjohn Company (Kalamazoo, MI), respectively. DAMGO (Peninsula Laboratories, Inc., Belmont, CA) and nor-BNI (Research Biochemicals Inc., Natick, MA) were purchased. Drugs were dissolved in saline in a fixed volume such that each rat received 5 μl of drug solution per injection. Saline alone in the same volume was injected as a vehicle.

Animal preparation. Male Sprague-Dawley rats (350–400 g; Zivic Miller, Pittsburgh, PA) were housed in a room with controlled temperature (24°C), humidity (50%) and light-dark cycles (lights on from 6:00 A.M.–6:00 P.M.) for at least 2 weeks before experiments. Food and water were provided *ad libitum*.

On the day preceding experiments, rats were anesthetized with ketamine hydrochloride (70 mg/kg i.m.) and xylazine (6 mg/kg i.m.) for cannulation of the right lateral cerebral ventricle, the tail artery and the external jugular vein. For i.c.v. drug injections, a 27-gauge needle attached to 5 cm of polyethylene tubing (PE-20) was implanted into the right lateral cerebral ventricle and fixed to the skull with dental cement. Coordinates for implantation into the lateral ventricle were 2 mm caudal to the bregma, 2 mm lateral to the midsaggital suture and 4 mm deep from the skull surface. After exposure by blunt dissection, both the tail artery and the external jugular vein were cannulated with PE-50 catheters. The tail artery cannula was used to monitor heart rate and mean arterial blood pressure and to withdraw arterial blood samples for arterial blood gas analysis. The jugular cannula was advanced into the superior vena cava to monitor respiratory rate *via* changes in intrathoracic pressure. These cannulae were directed s.c. to emerge at the back of the neck and extend from the cage through a protective wire spring. Arterial and venous cannulae were kept patent with twice daily 0.5-ml injections of a solution of heparin sodium (100 U/ml).

Assessment of cardiorespiratory function. The jugular catheter was connected to a microtransducer (Physiological Pressure Transducer, Narco Bio-systems, Houston, TX) for the monitoring of intrathoracic pressure which is synchronized with ventilatory movement of the chest. Intrathoracic pressure changes were recorded on a multichannel recorder (Lineacorder Mark VIIW/R301, Western Graphtec, Inc., Irvine, CA) and were manually counted in order to obtain respiratory rates. The tail artery catheter was connected to another microtransducer which interfaced with a Cardiovascular Analyzer (Buxco Electronics, Sharon, CT) and a personal computer for measurement of heart rates and mean arterial pressures.

Arterial blood samples (300 μl) were collected through the tail artery catheter into heparinized syringes. The pH, PO_2 (PaO_2) and PCO_2 ($PaCO_2$) of arterial blood samples were analyzed by a blood gas analyzer (System 1306 pH/blood gas analyzer, Instrumentation Laboratory, Lexington, MA). To minimize possible cardiovascular changes induced by the repeated blood withdrawal, immediately after their collection blood samples were replaced with an equal volume of a 5% dextran solution injected through the jugular catheter.

Experimental protocol. Rats remained individually housed in their home cages throughout experiments. After 2 hr of acclimation to surroundings and stable breathing, base-line respiratory rates, heart rates and mean arterial pressures were measured and 300 μl of arterial blood were collected for arterial blood gas analysis. Immediately after these measurements, drugs were administered. All compounds were dissolved in saline and were injected i.c.v. in a 5-μl volume followed by a 3-μl cannula flush. Saline in the same volume was injected in control rats. The time-effect characteristics of the responses to each drug dose or drug combination were monitored and established in individual rats. Dose-response and time course characteristics for each opioid ligand were determined in the initial experiments. In the subsequent experiments designed to evaluate *mu-kappa* interactions, nor-BNI and U-50,488H were injected 30 and 15 min before the administration of the *mu* agonists, respectively.

Data analysis. Statistical analysis of data was accomplished by analysis of variance for repeated measures and significant differences among treatment groups were identified by the Newman-Keul's test.

Results

Cardiorespiratory effects of *mu* agonists. The effects of DAMGO on cardiovascular and respiratory function are shown in figure 1, A-F. The i.c.v. injection of 0.6–10 nmol of DAMGO produced the dose-dependent development of acidosis (fig. 1A), hypoxemia (fig. 1B) and hypercapnia (fig. 1C). In addition, the 1.2-nmol dose of DAMGO induced a transient elevation in respiratory rate through a 20-min postinjection (interval). DAMGO significantly increased mean arterial pressure (fig. 1E; 1.2, 2.5 and 10 nmol) and decreased heart rate (fig. 1F; 2.5 and 10 nmol). All responses occurred within 10 min of DAMGO injection and persisted through at least a 30-min postinjection.

The respiratory and cardiovascular responses to morphine were qualitatively quite similar to those seen with DAMGO (fig. 2, A-F). In addition to significantly lowering blood pH (fig. 2A) and PaO_2 (fig. 2B) and increasing blood $PaCO_2$ (fig. 2C), 20- and 30-nmol doses of morphine significantly elevated respiratory rate (fig. 2D). Mean arterial pressure was increased

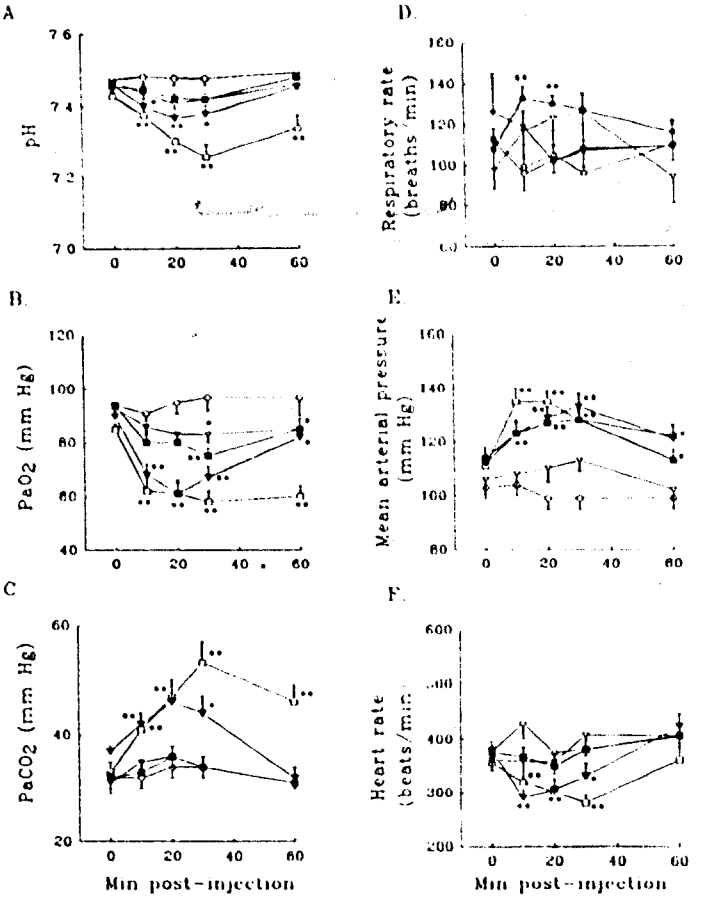

Fig. 1. A-F, effects of i.c.v. DAMGO on cardiorespiratory parameters in conscious rats. Data are presented as means ± S.E.M. for dose-treatment groups of six to nine rats. ○, vehicle; ▽, DAMGO (0.6 nmol); ■, DAMGO (1.2 nmol); ▼, DAMGO (2.5 nmol); □, DAMGO (10 nmol). *$P < .05$; **$P < .01$ when compared to vehicle-injected rats.

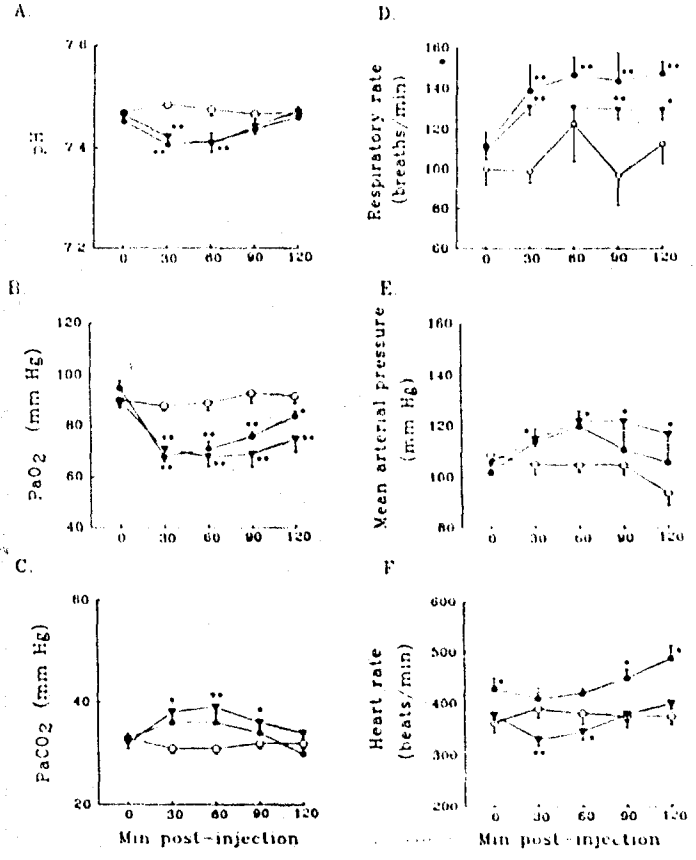

Fig. 2. A-F, effects of i.c.v. morphine on cardiorespiratory parameters in conscious rats. Data are presented as means ± S.E.M. for dose-treatment groups of 9 to 11 rats. O, vehicle; ●, morphine (20 nmol); ▼, morphine (30 nmol). *P < .05, **P < .01 when compared to vehicle-injected rats

and heart rate was decreased by 30 nmol of morphine, whereas paradoxically rats treated with 20 nmol of morphine instead had pressor responses that were accompanied by a delayed tachycardia (fig. 2, E and F). All responses to i.c.v. morphine were slower in onset and were more persistent than were seen with i.c.v. DAMGO and generally lasted through at least a 120-min postinjection interval.

***Kappa* ligand interactions with *mu* agonist effects on respiration.** As seen in table 1, respiratory and cardiovascular parameters were unaffected by either the *kappa* opioid agonist U-50,488H (20 and 200 nmol i.c.v.) or the *kappa* opioid antagonist nor-BNI (25 nmol i.c.v.). Although without measurable effects themselves, pretreatment with these ligands did significantly influence subsequent responses to both DAMGO and morphine. The interaction of U-50,488H with the effects of DAMGO on respiratory function is shown in figure 3. In the control rats pretreated with saline vehicle, as was seen in the initial experiments (figs. 1 and 2), the 2.5-nmol i.c.v. dose of DAMGO induced acidosis (fig. 1A), hypoxemia (fig. 1B) and hypercapnemia (fig. 1C), without significantly altering respiratory rate (results not shown). In contrast, in rats pretreated with U-50,488H (200 nmol i.c.v.) the subsequent effects of DAMGO on arterial pH, PaO_2 and $PaCO_2$ were substantially eliminated (fig. 3). Although occurring over a more prolonged time course, the effect of U-50,488H on morphine-induced respiratory depression was quite similar to that seen with DAMGO. Specifically, as shown in figures 4A through 4C, 200 nmol of U-50,488H significantly antagonized morphine-induced acidosis, hypercapnemia and hypoxemia. As was seen in the initial dose-response experiments, i.c.v. morphine also significantly increased respiratory rate; however, in contrast to the other respiratory parameters, U-50,488H did not inhibit this response to morphine (results not shown). These modulatory effects of U-50,488H appeared to be dose-dependent in that i.c.v. pretreatment with 20 or 63 nmol of U-50,488H did not consistently alter subsequent responses to either DAMGO or morphine (results not shown).

The *kappa* opioid antagonist nor-BNI (25 nmol i.c.v.) did not by itself alter subsequent respiratory responses to DAMGO, but it did substantially eliminate the modulatory effects of U-50,488H on DAMGO-induced respiratory depression (fig. 5). Specifically, relative to the rats injected initially with saline vehicle, rats administered nor-BNI (25 nmol i.c.v.) 15 min before injection of U-50,488H (200 nmol i.c.v.) had restored responses to a subsequent injection of DAMGO (2.5 nmol i.c.v.). Thus, the *kappa* opioid antagonist nor-BNI blocked the inhibitory influence of the *kappa* opioid agonist U-50,488H on the respiratory depressant actions of the *mu* agonist DAMGO.

Discussion

The present results confirm that, through actions within the central nervous system, the highly *mu*-selective opioid agonist DAMGO depresses respiration and increases mean arterial pressure in a fashion similar to morphine, albeit with slightly different time courses. In contrast, by themselves the *kappa*-selective ligands U-50,488H and nor-BNI failed to significantly alter any of the cardiorespiratory parameters monitored after i.c.v. drug injection. These results support the conclusions drawn from a number of earlier studies in which *mu* (but not *kappa*) opioid receptors were consistently identified as being primarily responsible for the central mediation of the respiratory depressant effects of morphine and other opioid analgesics (Yeadon and Kitchen, 1989; Shook *et al.*, 1989). Although there are also some indications that *delta* opioid receptors might contribute to a lesser degree to the respiratory depressant effects of opioid analgesics, these results have been less clear-cut due in part to variable outcomes, incomplete respiratory assessments and limited receptor ligand selectivities in a number of these studies (Holaday, 1982; Pazos and Florez, 1983, 1984). Regardless of these previous arguments concerning relatively greater or lesser roles of *mu*, *delta* and *kappa* receptors in the depression of respiration, it appears from the present and other collective results that a prominent (if not exclusive) role for *mu* opioid receptors can be postulated and, that along with their well-described antinociceptive effects, *mu* opioid agonists also clearly appear to be efficacious respiratory depressants in rats. Furthermore, the occasional disparities seen between drug effects on blood gases and respiratory rates underscore previously stated concerns regarding the accuracy and utility of the latter endpoint as a measure of respiratory status (Yeadon and Kitchen, 1989).

Several laboratories have provided evidence to suggest that the respiratory depressant effects of morphine and related narcotic analgesics are not mediated by the same class of receptors that is responsible for the antinociceptive actions of these drugs (McGilliard and Takemori, 1978; Ward and Takemori, 1983; Ling *et al.*, 1983; Wood *et al.*, 1982). For example, naloxonazine has been shown to selectively block the analgesic but not the respiratory depressant actions of morphine, prompt-

TABLE 1

Effect of U-50,488H and nor-BNI on cardiorespiratory function after i.c.v. injection in conscious rats

Values are expressed as means ± S.E.M. for each treatment group. NR, not recorded.

Parameter	Time	Treatment				
		U-50,488H			nor-BNI	
		Vehicle (n = 8)	20 nmol (n = 8)	200 nmol (n = 8)	Vehicle (n = 9)	25 nmol (n = 6)
Arterial pH	Pretreatment	7.475 ± 0.015	7.470 ± 0.010	7.437 ± 0.012	7.469 ± 0.007	7.466 ± 0.006
	30 min	7.477 ± 0.018	7.481 ± 0.014	7.456 ± 0.008	7.463 ± 0.012	7.466 ± 0.010
	60 min	7.490 ± 0.014	7.468 ± 0.019	7.454 ± 0.013	7.476 ± 0.012	7.459 ± 0.013
PaO_2[a]	Pretreatment	94 ± 4	88 ± 1	87 ± 1	90 ± 3	89 ± 1
	30 min	95 ± 6	92 ± 3	86 ± 2	88 ± 2	96 ± 5
	60 min	97 ± 7	89 ± 3	87 ± 1	89 ± 3	100 ± 3
$PaCO_2$[a]	Pretreatment	32 ± 2	31 ± 1	34 ± 1	33 ± 1	34 ± 1
	30 min	34 ± 2	31 ± 1	32 ± 1	31 ± 1	32 ± 1
	60 min	31 ± 1	31 ± 1	32 ± 1	31 ± 1	33 ± 1
Repiratory rate[b]	Pretreatment	112 ± 14	104 ± 7	121 ± 15	NR	NR
	30 min	107 ± 10	111 ± 8	121 ± 10	NR	NR
	60 min	109 ± 9	111 ± 8	111 ± 14	NR	NR
Heart rate[c]	Pretreatment	377 ± 19	368 ± 5	344 ± 15	NR	NR
	30 min	396 ± 19	368 ± 8	366 ± 18	NR	NR
	60 min	404 ± 19	379 ± 15	372 ± 19	NR	NR
Mean arterial pressure[a]	Pretreatment	103 ± 4	103 ± 4	126 ± 12	NR	NR
	30 min	107 ± 4	104 ± 3	126 ± 10	NR	NR
	60 min	99 ± 4	102 ± 3	123 ± 10	NR	NR

[a] Millimeters of mercury.
[b] Breaths per minute.
[c] Beats per minute.

ing the interpretation that naloxonazine-insensitive *mu*-2 receptors mediate respiratory depression whereas naloxonazine-sensitive *mu*-1 receptors mediate antinociceptive responses to morphine and related opioid analgesics (Ling *et al.*, 1983, 1985). In a closely related study, Wood *et al.* (1982) used lethality as a measure of respiratory depression and showed conversely that the *kappa* agonists EKC and MR 2034 selectively antagonized morphine-induced respiratory depression (as well as several other putatively *mu*-2-mediated neurochemical effects), without disrupting analgesia. These results preliminarily suggested that *kappa* opioid agonists might pharmacologically discriminate among putative *mu* isoreceptors and might thereby provide an approach to therapeutically dissociate *mu* receptor-mediated analgesia from respiratory depression side effects.

The blood gas alterations measured in the present study obviously reinforce aspects of the earlier findings of Wood *et al.* (1982) by clearly revealing that the more highly selective *kappa* opioid agonist U-50,488H also significantly antagonizes the respiratory depression resulting from i.c.v. administration of either morphine or DAMGO. However, because this interactive effect has by now been shown to also extend to a number of other *in vivo* opioid responses (including antinociception), the potential therapeutic utility of *kappa-mu* opioid interactions, with regard to the selective elimination of respiratory depression, is less immediately obvious. Specifically, *kappa* agonists such as U-50,488H have been consistently recognized to act as *mu* antagonists in a variety of *in vivo* preparations, including rat bladder motility (Sheldon *et al.*, 1987, 1989), antinociception (Ramarao *et al.*, 1988; Bhargava *et al.*, 1989) and seizure threshold models (Tortella and Holaday, 1986; Porreca and Tortella, 1987).

In these more recent studies, *mu* opioid agonists have been categorized on the basis of their sensitivity to this antagonism. For example, in the bladder motility preparation, the *kappa* opioid agonists U-50,488H, dynorphin A, EKC and tifluadom consistently antagonized responses to i.c.v. administration of the *mu* agonists morphine and normorphine, whereas responses to several other *mu* agonists such as DAMGO, PL017, phenazocine or meperidine were consistently unaffected by these *kappa* compounds (Sheldon *et al.*, 1987, 1989). Similarly, Porreca and Tortella (1987) showed that U-50,488H, in doses lacking agonist effects, antagonized etorphine but not DAMGO in both bladder motility and flurothyl seizure threshold models.

The cellular mechanisms mediating this differential antagonism are presently unclear. As discussed by these investigators, variations in the relative receptor affinities (and potencies) of the different *mu* agonists used in these studies might conceivably render them differentially sensitive to antagonism by *kappa* agonists. Alternatively, subtypes of *mu* opioid receptors within the central nervous system might be proposed to explain these patterns. Because *kappa* agonists have been postulated to act as antagonists at *mu*-2 opioid receptors (Wood *et al.*, 1982), one might suspect that morphine and normorphine predominantly exert their central effects on the bladder through actions at *mu*-2 receptors, whereas DAMGO, PL017 and phenazocine produce similar effects through actions at a different *mu* receptor site, such as the putative *mu*-1 receptor. However, as pointed out by Sheldon *et al.* (1987), inasmuch as bladder effects of i.c.v. morphine, DAMGO and D-Pen^{2}D-Pen^5-enkephalin can all be antagonized by naloxonazine pretreatment, it is unlikely that the conventional *mu*-1 and *mu*-2 isoreceptor definition applies to the *mu* receptor distinctions made with *kappa* agonists in this particular *in vivo* preparation. Moreover, Pasternak and colleagues (Pasternak and Wood, 1986) have distinguished *mu* isoreceptors *in vivo* largely on the basis of differing naloxonazine-sensitive and -insensitive pharmacological responses, whereas these latter postulated isoreceptor distinctions are seen within groups of ligands producing identical shared responses. Regardless of the underlying mechanism, differential *kappa* sensitivity was not observed in the present study with respiratory depression as a pharmacological endpoint.

The antagonistic effects of U-50,488H upon both morphine and DAMGO-induced respiratory depression apparently in-

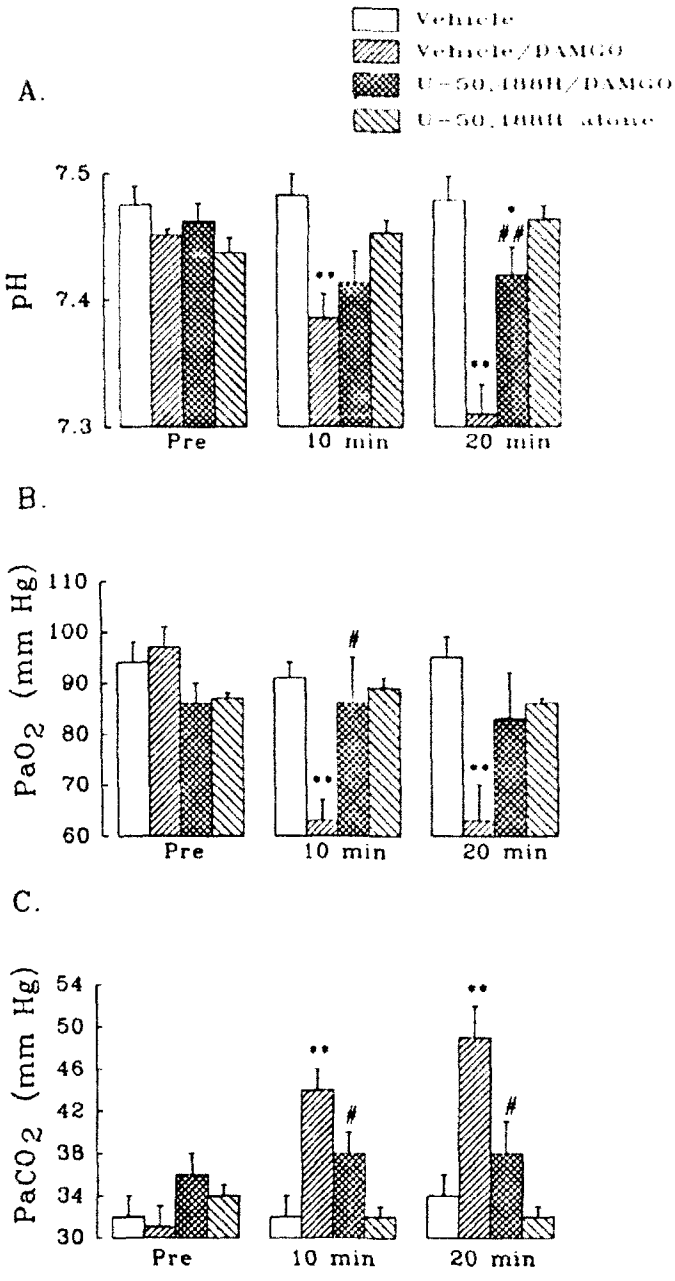

Fig. 3. A-C, effects of U-50,488H on DAMGO-induced alterations in respiratory function. Six rats were pretreated i.c.v. with saline vehicle and nine rats received pretreatment (Pre) with 200 nmol of U-50,488H 15 min before i.c.v. injection of 2.5 nmol of DAMGO. U-50,488H attenuated the acidosis, hypoxemia and hypercapnia induced by DAMGO. Data are expressed as means ± S.E.M. *P < .05; **P < .01 in comparison with base-line values. #P < .05; ##P < .01 in comparison with DAMGO-injected rats pretreated with saline vehicle. Lower doses of U-50,488H (20 and 63 nmol failed to alter subsequent responses to 2.5 nmol of DAMGO (results not shown).

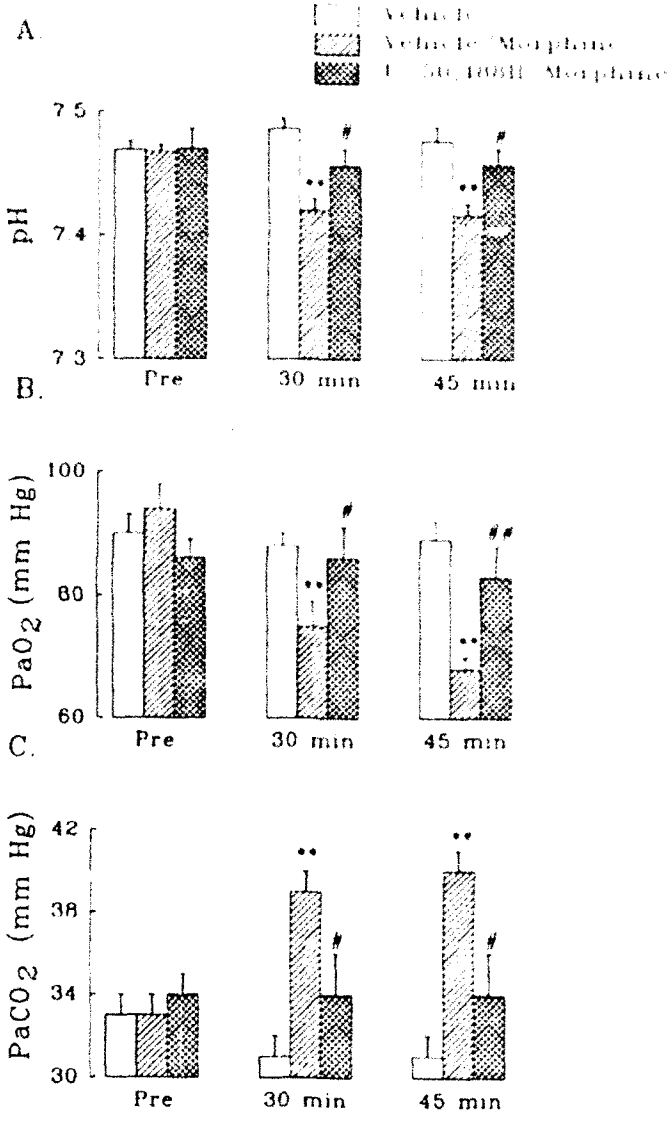

Fig. 4. A-C, effects of U-50,488H on morphine-induced alterations in respiratory function. Eleven rats were pretreated (Pre) i.c.v. with saline vehicle and nine rats received i.c.v. Pre with 200 nmol of U-50,488H 15 min before i.c.v. injection of 30 nmol of morphine. U-50,488H attenuated the acidosis, hypoxemia and hypercapnia induced by morphine between 30- and 60-min postinjection. Data are expressed as means S.E.M. **P < .01 when compared to base-line data. #P < .05, ##P < .01 when compared to morphine-injected rats Pre with saline vehicle. Lower doses of U-50,488H (20 and 63 nmol failed to alter subsequent responses to 30 nmol of morphine (results not shown).

volved interactions with *kappa* opioid receptors, because in both cases they were blocked by pretreatment with the *kappa* antagonist nor-BNI. The antagonism of the respiratory depressant effects of DAMGO argues that the previously described distinctions of *mu* opioid agonists based upon their sensitivity to U-50,488H are relative and not absolute. It is possible that, if they lacked overt agonist activity in these other preparations, higher doses of U-50,488H and other *kappa* agonists might have antagonized the unresponsive *mu* effects of DAMGO and other ligands in earlier studies, particularly when one considers that, with a dose of U-50,488H comparable to that used in the earlier studies, we failed to see antagonism of either morphine- or DAMGO-induced respiratory depression. In fact, Porreca and co-workers have also noted that to some degree the interactive patterns they describe are not absolute in the sense that, whereas several *kappa* opioid agonists could block morphine and normorphine bladder motility effects, only U-50,488H could block the identical effects of etorphine and sufentanil (Sheldon *et al.*, 1987).

Because the antagonism of the respiratory depressant effects of these *mu* opioid agonists by U-50,488H appears to be *kappa*

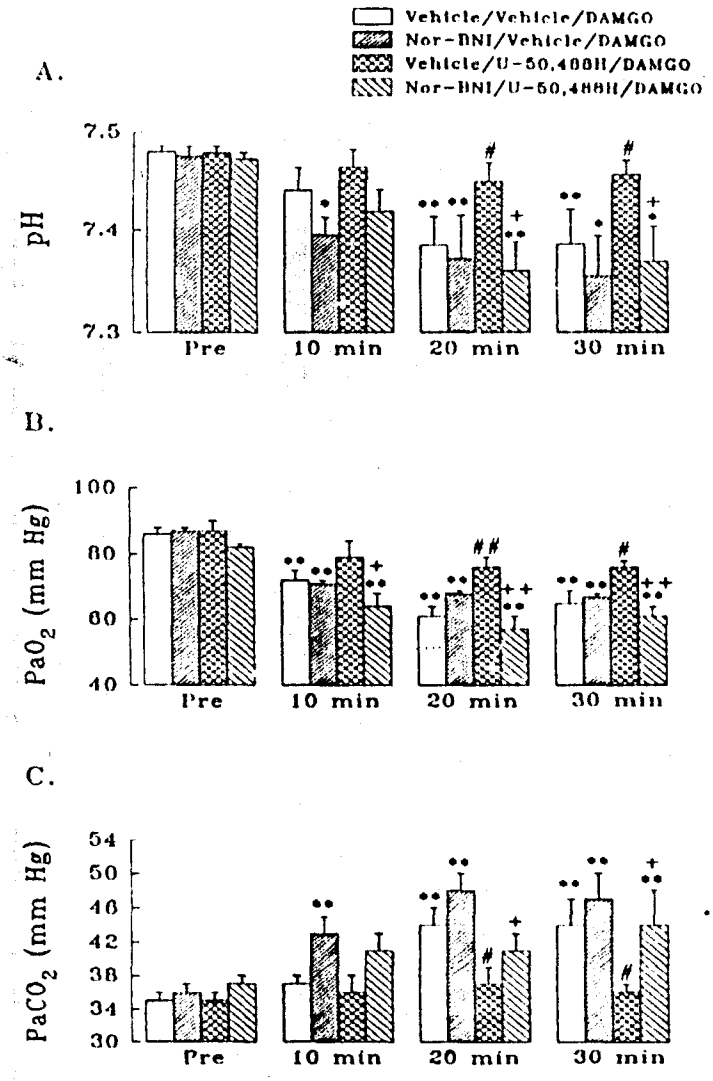

Fig. 5. A-C, nor-BNI blocks the *mu*-antagonistic actions of i.c.v. U-50,488H. Saline vehicle or 25 nmol of nor-BNI were administered 15 min before an i.c.v. injection of saline or U-50,488H (200 nmol). Fifteen minutes after U50,488 injection, rats received i.c.v. injections of 2.5 nmol of DAMGO. Nor-BNI reversed the *mu* antagonistic effects of U-50,488H on respiratory function. Values are presented as means S.E.M. for groups of seven and nine rats. *P <.05; **P < .01 when compared to respective base-line values; #P .05; ##P < .01 when compared to DAMGO-treated rats preinjected (Pre) with saline vehicle only (vehicle-vehicle-DAMGO); +P < .05; ++P < .01 when compared to DAMGO-treated rats Pre with only U-50,488H (vehicle-U-50,488H-DAMGO).

receptor-mediated, actions at these two receptor types seem to be somehow interactively coupled through an as yet unidentified convergence in opioid receptor effector mechanisms. The requirement for higher doses of U-50,488H for *mu* antagonism in the present study (200 nmol rather than the approximately 20-nmol dose of U-50,488H used in the aforementioned studies) was paralleled by similar requirements for greater doses of morphine and DAMGO to elicit respiratory depressant responses and might reflect an overall need for greater total receptor occupancy by morphine and other *mu* agonists to produce an appreciable depression of respiration. If so, it can be further reasoned that, through a *kappa-mu* receptor interactive coupling mechanism, a greater concentration of *kappa* agonist was required to antagonize the greater administered doses of morphine or DAMGO that were presumably occupying larger numbers of "*kappa*-sensitive" *mu* opioid receptors.

The utility of using antagonistic *kappa-mu* opioid interactions as a therapeutic approach to dissociate analgesic and respiratory depressant actions of opioid analgesics obviously hinges on the selective impact of these interactions on respiration and not analgesia. *Kappa* opioid agonists, in addition to having antinociceptive actions by themselves (Porreca *et al.*, 1987), have also been shown to differentially influence *mu* opioid-induced antinociception in morphine-naive and morphine-tolerant rats (Tulunay *et al.*, 1981; Ramarao *et al.*, 1988; Bhargava *et al.*, 1989), with antagonism seen in the former and potentiation seen in the latter. A similar loss of the antagonistic activity of *kappa* opioids after chronic morphine treatment has been described in a squirrel monkey shock titration procedure by Craft and Dykstra (1992). Whether *kappa* opioid antagonism of morphine's respiratory depressant effects remains intact in morphine-tolerant rats, or is similarly differentially influenced, is presently uncertain. Clearly, future parallel assessments of the impact of *kappa* agonists on respiratory depressant actions of morphine-related analgesics in tolerant animals will serve to distinguish the viability of this pharmacological strategy for patients receiving chronic opioid therapy.

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